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By: (Reg # 38515)

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PATENT
Attorney Docket No.: 16336-000300US
Client Ref. No.: 1756-049455-1331PT

# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| In re application of: | Examiner: D. Clark |
| William R. A. Osborne, et al. | Art Unit: 1633 |
| Application No.: 08/217,324 | |
| Filed: March 24, 1994 | **DECLARATION OF WILLIAM R. A. OSBORNE UNDER 37 CFR 1.132** |
| For: DEVICES AND METHODS FOR IMPLANTING TRANSDUCED CELLS | |

Considered DW 5/11/01

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, William R. A. Osborne, declare and state as follows:

1. I am an inventor in the above-identified patent application.

2. I am presently employed as a Research Professor in the Department of Pediatrics at the University of Washington. My Curriculum Vitae is attached hereto.

3. I have read and fully understand the specification and claims of the above-identified patent application, as well as all Amendments to the claims and accompanying remarks filed in the application.

4. All of the experiments described in this Declaration were performed and evaluated by me or under my direct instruction and supervision.

5. In this Declaration, I have provided detailed comments on the teachings of the "prior art" references cited by the Patent Office as they relate to the invention claimed in the present application. For this purpose, I read the outstanding Office Action identified as Paper No. 26, as well as the previous Office Actions (Papers No. 6, 11, 15, and 19) presented in the application. I

also read and fully understand the references cited in the current and previous Office Actions, particularly including the Zalewski et al. (WO 93/15609) Nabel et al. (U.S. 5,328,470) and Anderson et al. (WO 90/224,525) references. I have analyzed these references with the goal of ascertaining their individual teachings and to determine whether these references, collectively, "teach or suggest" the presently claimed invention.

6. My conclusion, based on the factual considerations summarized below, is that the Zalewski et al. (WO 93/15609), Nabel et al. (U.S. 5,328,470), and Anderson et al. (WO 90/224,525) references, taken for what they teach as a whole, fail to provide sufficient motivation and direction that would have led a person of ordinary skill in the art at the time of the invention to make and practice the invention as claimed.

7. I further conclude based on the facts presented herein that the results disclosed for the invention claimed in the instant application, as detailed in the specification and further clarified by the experimental data set forth herein below, represent "unexpected results" in view of the prior art of record in the application. Thus, even if it was presumed that the Zalewski et al., Nabel et al., and Anderson et al. references collectively "suggest" the presently claimed invention, these references would fail to provide "a reasonable expectation of success" for practicing the invention as disclosed in the specification.

8. The Examiner has repeatedly cited the Zalewski et al. reference as allegedly teaching certain aspects of the invention, which are characterized to include:

> methods and kits with devices employing interferon gene therapy for the treatment of vascular disorders. More specifically, the references discloses transformation of smooth muscle cells on page 8 using various gene transfer methodologies. Further, page 9 of the reference discloses the use of implant devices to hold and contain said vascular smooth muscle cells. (Office Action Paper No. 11, at p. 10).

9. My interpretation of this reference differs substantially from the foregoing interpretation. To begin with the Zalewski et al. reference focuses solely on *in situ* transduction methods for vascular gene therapy. There is no disclosure whatsoever of any "implant devices to hold and contain said vascular smooth muscle cells." The only devices and methods taught by

Zalewski et al. are "injection and transcatheter delivery devices to deliver a solution" or a "perfusate" under pressure, containing genes and vectors to transduce smooth muscle cells in arteries *in situ* (see, e.g. page 8, lines 21-23; page 9, lines 14-35, page 10, lines 4-8). The proposed use of these devices is transitory, lasting 1-2 minutes. This limited window of time for insertion of the perfusion catheter is based on the fact that the device occludes the target vessel, which presents a concomitant risk of myocardial infarction (see, e.g. page 10, lines 4-8). There is therefore no disclosure or suggestion of devices or methods "to hold and contain" transduced smooth muscle cells, particularly in the manner of the presently claimed prosthetic devices which are seeded with cells *ex vivo*, which cells are previously transduced *ex vivo*, and implanted as a long term graft. In fact, the Zalewski et al. reference teaches away from long-term graft devices by disclosing perfusion catheters which are taught as delivery vehicles for solutions and perfusates, not cells, and which function only on a very transient delivery basis. In contrast, the presently claimed devices and methods deliver transduced SMCs integrated in a prosthetic implant that physically replaces or bypasses existing vessels.

10. The teachings of Zalewski et al. <u>focus the artisan's attention on *in situ* transduction methods for vascular gene therapy</u>. This aspect of the reference strongly influences the "direction" and extent of "motivation" that a skilled artisan would have gleaned from the reference to embark on a course leading toward, or away from, the presently claimed invention. From my analysis of the facts, I conclude that the Zalewski et al. reference <u>teaches directly away from long-term delivery engrafting devices and methods as presently claimed</u>.

11. The focus of Zalewski et al. on *in situ* transduction devices and methods are supplemented by numerous additional publications in the literature which similarly depart in the fundamental course of their teachings from the devices and methods of the present invention. These reports include a considerable assemblage of articles that expressly favor *in situ* over *ex vivo* transduction methods for implementing vascular gene therapy. Among these reports is the article by Nabel et al., <u>Science</u> <u>249</u>:1285-1288, 1990 (of record), which appears to refute the proposed utility of implanting in vitro transduced endothelial cells mentioned as an alternative gene therapy method in the Nabel et al. patent (U.S. Patent No. 5,328,470) and specifically cited by the Examiner. In this article, Nabel and coworkers expressly criticize their earlier vascular gene therapy studies that

focused on *ex vivo* transduction and reimplantation of genetically modified endothelial cells. In relevant part, the authors state that:

> Because these studies required that syngeneic cell lines be established before genetic modification, adaptation to the treatment of human disease remained cumbersome. We now report that a recombinant gene can be efficiently expressed at a specific site in vivo by direct introduction of genetic material at the time of catheterization. (page 1285, middle and right columns, emphasis added).

12. Similar teachings are in fact provided in the Nabel et al. patent cited by the Office, wherein the inventors point to a distinct advantage that can be achieved through "introduction of recombinant genes directly" *in vivo* (as opposed to *ex vivo* transduction). The Nabel et al. patent disclosure involving direct gene transfer (for cancer treatment) reportedly supplants "traditional" (*in vitro*) gene transfer techniques, as emphasized in the following passage:

> The prior art approaches (referring specifically to "modification of tumor cells in vitro followed by transfer of the modified cells") are disadvantageous because they subject the cells to selection in different growth conditions from those which act in vivo, and because they also require that cell lines be established for each malignancy, thereby rendering adaptability to human disease considerably more difficult. (column 12, lines 34-45, underscoring added to relevant text).

13. These teachings lead directly away from *ex vivo* transduction of SMCs and *ex vivo* seeding of transduced SMCs onto a prosthetic vascular implant, as presently claimed. There is an express teaching against *ex vivo* transduction techniques. At the same time, there is an implicit teaching against the use of any kind of implantation device or method to introduce transduced cells for vascular gene therapy. This skepticism forecast by Nabel et al. with regard to "traditional" *in vitro* gene therapy methods reflects a widely adopted perspective in the art at the time of the invention. This perspective, expressly favoring direct, *in vivo* transduction methods for vascular gene therapy, proved to be strongly influential in the art, as evinced by more recent reports. For example, Kuo et al., Am. J. Roentgenology 171:553-558, 1998 (copy enclosed), teach directly away from *ex vivo* transduction and implantation devices for vascular gene therapy, as follows:

> The most advanced tissue engineering strategies currently available are cell-based in vitro studies or simplistic ex vivo strategies. These

> strategies are by their very nature inefficient, somewhat awkward, and thus of limited clinical usefulness . . ..
>
> By transfecting the desired vein segment with the Adv/RSV-tPA construct in situ, we were able to confer the desired thrombolytic characteristics to the graft in vivo, avoiding the need for complex ex vivo or in vitro treatments. (page 556, emphasis added).

14. Considering the foregoing evidence, there does not appear to be a scientifically well-founded "suggestion" or "motivation" provided by the cited references, taken as a whole, that would have led the artisan to independently create the presently claimed devices and methods. Like Zalewski et al., Nabel et al. clearly teach that *in situ* transduction methods are favored, leading directly away from the combination recited in the claims. At the same time, the exact combination of teachings presently relied by the Examiner remains unclear from the record. As discussed above, Zalewski et al. appears to been originally cited in error, for allegedly teaching "the use of implant devices to hold and contain said vascular smooth muscle cells." (Office Action Paper No. 11, at p. 10). In the present Office Action, the Examiner appears to acknowledge this error, stating that:

> The fact that the Zalewski et al. reference does not specifically disclose an implantable prosthetic device lined with SMC (smooth muscle cells) does not take away from the fact that the Nabel reference does. (Paper No. 26, at page 4, underscore added).

15. This excerpt from the Office Action raises a new puzzle as to the Examiner's interpretation of Nabel et al. As is clearly noted above, the teachings of Nabel et al. are expressly limited to the use of "catheter means" "for the instillation of vectors or cells" (see, e.g., columns 7 and 8). Even when cells are transduced ex vivo, Nabel does not teach the use of an "implantable prosthetic device" that is "lined with" transduced cells. On the contrary, in column 7 of Nabel et al. the specification teaches that: "After instillation of the infected endothelial cells, the balloon catheter is removed . . .." Accordingly, the reference must be considered to teach directly away from "implantable device lined with SMC" or any other kind of cells, as further discussed above. This teaching away from the invention is further underscored in the subsequent Science article by Nabel et al. (supra, at page 1286), which criticized the authors' prior method employing a catheter to instill *ex vivo* transduced endothelial cells:

> Although this method was effective, it required that cells syngeneic to the recipient animal be prepared and transduced, which took several weeks to prepare. Direct retroviral infection and liposome transfection allow the introduction of recombinant genes into any site accessible to a catheter without advanced preparation . . . this approach minimizes potential complications . . .. (emphasis added).

16. On this basis, it is apparent that the Nabel et al. and Zalewski et al. cannot be relied upon as teaching or suggesting *ex vivo* vascular cell transduction and, separately or in combination, the use of a prosthetic implant lined with transduced cells. In fact, both references clearly teach away from both aspects of the present invention, as well as the proposed combination of these features. With respect to the Anderson et al. publication (WO 90/224,525), the Examiner correctly notes that this reference is limited in its description to using "genetically engineered endothelial cells and the use thereof for expressing a therapeutic agent" (Anderson et al., at p. 1, Office Action Paper No. 26 at page 4, underscore added).

17. As a preliminary point in discussing the teachings of Anderson et al., it should be noted that, although the reference describes "a blood vessel graft which includes genetically engineered endothelial cells" (see, e.g., page 6), the reference actually purports to provide a large array of useful, alternative "solid supports" for transduced endothelial cells. These alternative supports include vascular shunts and by-passes, pads, strips, gels and other compatible implants (see, e.g., page 5). In my opinion, this broad spectrum of allegedly useful "implant" devices detracts from any proposed teaching that relates to the presently claimed vascular grafts.

18. More importantly, the actual "Examples" provided by Anderson et al. relating to the present invention are limited to a brief description and evaluation of transduced endothelial cells in culture. The only Example that pertains directly to a seeded "graft" is a limited, *in vitro* Example wherein the seeded graft was maintained and assayed strictly in a tube of culture medium with no evidence of *in vivo* viability or transgene expression (see, Example 1, e.g., at page 12). This limited teaching does not, in my interpretation, support the Examiner's contention that Anderson et al.:

> discloses to the skilled artisan a vascular graft coated with genetically modified autologous endothelial cells, and further discloses the use of

> this invention to deliver erythropoietin, Factor IX, G-CSF and GM-CSF proteins, among others. (Paper No. 26, at page 4). (underscore added).

19. More specifically, I do not believe that the Anderson et al. reference would have been interpreted by the skilled artisan as providing an effective vascular graft for gene therapy in the manner alleged by the Examiner. I am also puzzled as to how the Examiner's interpretation of Anderson et al. is reconciled with the Examiner's own rejections and technical concerns raised in the Office Action under 35 U.S.C. § 112, first paragraph. Clarification of these issues is necessary to assist me in presenting *in vivo* gene therapy aspects of the present invention that have been temporarily withdrawn from consideration in the application in the accompanying Amendment.

20. The limited teachings of the Anderson et al. reference do not provide sufficient scientific motivation or guidance to overcome the negative teachings of Zalewski et al., Nabel et al., and others, noted above, that teach away from the use of *ex vivo* cellular transduction and instillation in any form, as well as the more unpredictable task of *ex vivo* graft seeding and implantation, in favor of direct, *in situ* transduction methods. More importantly, Anderson et al. provides nothing that would lead the artisan to substitute SMCs for the designated preferred, endothelial cell targets for transduction, seeding and/or implantation, allegedly described in the reference. Even if one skilled in the art accepted the teachings of Anderson et al. (considering the limited working examples noted above), to evince successful transduction, seeding and implantation of endothelial cell-coated vascular grafts, this acceptance would not translate to a "reasonable expectation of success" for extending these teachings to achieve the presently claimed vascular grafts incorporating transduced SMCs. This is particularly if one considers the distinct challenges and uncertainties involved in SMC and endothelial cell culture, transduction, seeding, and prolonged viability and transgene expression *in vivo*.

21. My reading of the prior art of record indicates that, even if Anderson et al. and other publications are accepted as teaching a successful vascular graft seeded with transduced endothelial cells suitable for long term implantation and expression of a foreign gene, there is no sufficient teaching or suggestion in the art that would have lead the ordinarily skilled practitioner, at the time of the invention, to substitute transduced SMCs for endothelial cells in a vascular graft

implant, as the Examiner proposes. There are instead numerous, independent grounds to support my conclusion that the art teaches directly against making the proposed substitution of cell types in a vascular graft.

22. As an initial point for consideration, if the device of Anderson et al. is actually useful in the manner advocated by the Examiner, it would be counterintuitive to substitute SMCs for endothelial cells to arrive at the presently claimed invention. It is a fundamental principal of scientific reasoning that one should not alter a proven device or system demonstrated to work for an intended purpose, absent some compelling, practical motivation to do so. It is another basic scientific tenet not to increase the complexity of a proven device or system, without some well-reasoned expectation of substantially improved results. Thus, if Anderson et al. in fact teaches a useful vascular graft seeded with transduced endothelial cells for long term implantation and expression, one would be directly countermotivated to substitute SMCs for endothelial cells in such a useful implant, contrary to the Examiner's suggestion. In this regard, it is noted that numerous other references in the record follow the same direction as allegedly taught by Anderson et al., by reporting transfection of endothelial cells, the use of endothelial cells to line vascular grafts, viability of endothelial cell-lined grafts in vivo, and/or development of vascular grafts seeded with transduced endothelial cells. In particular, the Examiner's attention is directed to Dichek et al., Circulation 80:1347-1353, 1989 (of record), and Flugelman, Thromb. Haemost. 74:406-410, 1995 (copy enclosed for consideration and entry in the record). Dichek et al. reports successful coating of retroviral-transduced endothelial cells onto stainless steel stents. The reference does not provide in vivo data, but instead cites Wilson et al. and Nabel et al. as having "reported encouraging data on the ability of implanted transduced endothelial cells to survive and proliferate in vivo." (page 1352, left column). Flugelman also teaches "the use of genetically engineered endothelial cells to improve the surface of a metallic endovascular prosthesis known as a stent." (page 406, right column).

23. In light of these and other reports, there does not appear to be any direct, practical suggestion or compelling motivation in the art of record to make the proposed substitution of SMCs for endothelial cells in a prosthetic vascular graft for in vivo gene therapy, as proposed by the Examiner. On the contrary, sound scientific reasoning, and a consensus of teachings noted above, would appear to direct otherwise. In this context, the evidence of record clearly discloses, or

is at least advocated by the Examiner to disclose, that: (1) endothelial cells are an "excellent target" for gene therapy, in part because they line the vascular lumen and thus provide the advantage of direct exposure (of the transduced cells and their secreted products) to the circulation; (2) endothelial cells are reportedly shown by Anderson et al. and others to be readily transduced and seeded onto vascular graft surfaces; and (3) seeded endothelial cells on vascular grafts have been demonstrated to exhibit long-term survival in vivo.

24. The following references and citations confirm the above-noted differences relating to the proposed use of SMCs as allegedly "obvious" substitutes for endothelial cells in gene therapy and, more specifically, in the context of transduced cell-seeded, vascular grafts for *in vivo* transgene expression. With regard to the prior art teaching a clear preference for endothelial cell targets in this context, Welling et al., Hum. Gene Ther. 7:1795-1802, 1996 (copy enclosed) states as follows:

> Endothelial cells are considered an excellent target for gene transfer because the represent a durable tissue located strategically at the blood tissue interface. A number of investigators have successfully transduced the endothelium of large muscular arteries (Nable and Nabel, 1994; Messina et al., 1995) (p. 1796, left column).

25. Nabel et al., U.S. Patent No. 5,328,470 (of record), teach that direct, *in situ* transduction of endothelial cells provides the following advantages:

> In this way, the recombinant genes may be secreted directly into the circulation which perfuse the involved tissue or may be synthesized directly within the organ. (column 5, lines 19-22).

26. Similarly, Zweibel et al., Science 243:220-222, 1989 (copy enclosed), teach that:

> The endothelium, because of its contiguity with the bloodstream, is a particularly attractive target for the delivery of functional genes in vivo. The use of endothelium for gene transfer would permit secretion of a recombinant protein from genetically engineered endothelial cells directly into the blood. (page 22, right column, underscore added).

27. Reiterating and affirming these teachings, Zwiebel and other coworkers stated in Biochem. Biophys. Res. Comm. 170:209-213, 1990 (copy enclosed) that:

> Endothelial cells are attractive targets for gene transfer because of their immediate contact with the bloodstream, and, therefore, they might serve as attractive targets for therapeutic drug delivery. . . .. The fact that a recombinant gene can be readily inserted and efficiently expressed into human endothelial cells suggests that these cells may be able to serve a role in human gene therapy. (page 209, Abstract, emphasis supplied).

28. Further validating these teachings, Wilson et al., Science 244:1344-1346, 1989 (of record), state that:

> Because of their proximity to the blood stream endothelial cells are an **obvious** candidate for delivering therapeutic proteins systemically. (page 1346, left column, emphasis added).

29. In direct contrast to these teachings, SMCs were not viewed in the art at the time of the invention to be an "excellent target" for gene therapy. Moreover, SMCs are not naturally in direct contact with the circulating blood, but are instead covered by endothelial cells that would at least impair the exposure of transduced SMCs to the circulation to yield a therapeutic influence following transgene expression. Finally, SMCs had not been shown at the time of the invention to be readily transduced and seeded onto vascular graft surfaces, nor had their by long-term survival as a transduced, seeded cell layer on vascular grafts been established.

30. Yet another important point to consider is that SMCs and endothelial cells would not have been viewed to be "interchangeable" in a vascular graft, i.e., whereby SMCs could be used as an equivalent substitute for endothelial cells. In other words SMCs would not have been considered "as an equivalent substitute for endothelial cells." Thus, to include SMCs as a component of a vascular graft as advocated by the Examiner, rather than simply substituting one cell type for the other to make the proposed combination, the artisan would need to assemble a combination of multiple cell types in the graft, rendering the proposed combination far more complex to engineer, and much less likely to succeed. This follows the teachings noted above that endothelial cells are an "excellent target" for gene therapy. In addition, there was a long-standing

consensus in the art at the time of the invention that endothelial cells are an important or essential component of vascular implants, to prevent thrombosis and other adverse effects and otherwise better mimic natural blood vessel structure/function. In this context, Welch et al., Ann. Vasc. Surg., 6:473-484, 1992 (of record) reviewed a broad spectrum of literature relating to endothelial vascular graft seeding, stating that:

> Attempts to improve prosthetic graft performance have progressed broadly along two fronts: mechanical and biological. The latter adopts the concept that improved performance could be achieved if the luminal surface of the graft had biological characteristics of normal vessels, being lined with endothelium capable of resisting platelet aggregation. (page 473, left column, emphasis added). . . .. These original experiments have since generated a large volume of research to develop a technique to line prosthetic grafts with a confluent functional endothelial cell monolayer. (id., right column).

31. Similarly, Vohra et al., Eur. J. Vasc. Surg. 5:93-103, 1991 (of record), teach that:

> In order to overcome the thrombogenecity of synthetic vascular prostheses, attempts have been made to line these grafts with living endothelium.[1-3] Animal studies have shown reduced platelet adhesion and improved patency in endothelial cell seeded grafts.[4-6] Dacron and Polytetrafluoroethylene have both been successfully seeded with endothelial cells resulting in confluent monolayers in vitro as well as in vivo.[2,3,6-9]

32. Interestingly, one of the principal benefits of endothelial cell seeding of vascular grafts has been considered to be the prevention of smooth muscle cell proliferation in grafts to prevent graft occlusion. This and related teachings in the art further direct the artisan away from incorporating seeded SMCs in vascular graft implants. Briefly, a principal drawback in vascular graft surgery is restenosis and other forms of neointimal hyperplasia mediated by excessive proliferation of SMCs at sites of vascular graft implantation. After the implantation of a vascular graft, aberrant recruitment and growth of SMCs commonly narrows or occludes the vessel lumen, leading to a loss of patency and/or graft failure. In light of these concerns, it is a widely proposed goal in the art to block SMC recruitment or growth at sites of surgical vascular injury, including vessel grafts. For example, Isner, U.S. Patent No. 5,830,879 (copy enclosed), teaches a method for

reendothelialization of the lining of an injured blood vessel using *in situ* transfection of DNA encoding vascular endothelial growth factor (VEGF). This stated purpose for this method is that it "inhibits smooth muscle cell proliferation and consequently reduces restenosis." (Abstract, underscore added). See, also, McCarthy, Lancet 347:752, 1996 (copy enclosed). This reference cites Isner's work involving in situ VEGF transduction of endothelial cells, specifying that the goal of the work is to both "stimulate endothelial proliferation" and "limit smooth-muscle-cell proliferation and other changes that cause restenosis."

33. Numerous other references teach away from using SMCs, or a combination of endothelial cells and SMCs, as a component for seeding a vascular prosthesis. Following the later course of development in the art, noted above (favoring direct, *in situ* transduction methods over *ex vivo* transduction and implantation) Mann et al., Proc. Nat. Acad. Sci. USA 92:4502-4506, 1995 (copy enclosed) teach a modified vascular bioprosthesis that is genetically engineered specifically to block SMC growth. Thus, at page 4502 (Abstract), Mann and colleagues teach that:

> an intraoperative gene therapy approach using antisense oligodeoxynucleotide blockage of medial smooth muscle cell proliferation can prevent the accelerated atherosclerosis that is responsible for autologous vein graft failure. (underscore added).

34. The articles noted above, that specifically teach seeding of transduced endothelial cells in vascular grafts to inhibit SMC proliferation are particularly relevant to the present analysis. These articles can only be considered to teach directly away from seeding grafts with SMCs, alone or in combination of endothelial cells and SMCs. In this context, Dichek et al., supra at page 1347 (Abstract) teaches that:

> The use of intravascular stents may be limited by both local thrombosis and restenosis due to intimal proliferation. In an effort to provide solutions to these problems, we seeded stents with genetically engineered endothelial cells . . . (for) improvement of stent function through localized delivery of anticoagulant, thrombolytic, or antiproliferative molecules. (underscores added).

35. Similarly, Wilson et al., WO 89/05345 (copy enclosed), teaches that:

> There are many advantages to endothelial cells of the present invention. For example, they can be designed to improve the characteristics of endothelial cell-lined prosthetic implants by enhancing or improving the ability of endothelial cells to seed or bind to the inner surface of the implant; by modifying the endothelial cells used to line an implant so that they will grow; or by overcoming the problem, encountered with presently-available implants, of smooth muscle cell growth at the implant ends, which results in narrowing, and, ultimately, closing off of the ends. (page 4, lines 11-20, emphasis added) . . . (for example by) secretion of an inhibitor of smooth muscle proliferation to prevent luminal stenosis due to smooth muscle hypertrophy. (page 5, lines 5-7, underscore added).

36. As stated above, these references teach directly away from seeding grafts with SMCs, alone or in combination with endothelial cells. The Wilson et al. reference, because it teaches methods for "improving the ability of endothelial cells to seed or bind to the inner surface of the implant", further teaches away from layering endothelial cells (i.e., in combination) over a graft initially seeded with SMCs.

37. In light of all of the foregoing evidence and remarks, I conclude that the art of record, taken for what it teaches as a whole, fails to provide sufficient motivation and direction that would have led a person of ordinary skill in the art, at the time of the present invention, to make and practice the invention as claimed.

38. I further conclude that, even if the art of record is considered to collectively "suggest" the presently claimed invention, the results disclosed in the specification for the invention represent "unexpected results." These results, as clarified by the experimental data set forth in the application and further expanded herein below, are not predicted with "a reasonable expectation of success" by the art of record. This conclusion is based on my detailed evaluation of the experimental results in the application and herein, and on my interpretation of the relevant references above.

39. Briefly, each of these basic embodiments of the invention presently set forth in the claims are supported by detailed, *in vitro* and *in vivo* examples, which demonstrate successful isolation, transduction and seeding of SMCs onto prosthetic vascular grafts, as well as long term

viability and continued transgene expression by the seeded SMCs. Example 1 of the specification describes isolation and characterization of SMCs (pp. 12-13) from a non-human primate (baboon), which cells are subsequently efficiently transduced and selected and shown to stably express an exemplary gene of interest (pp. 13-15). The selected, transduced cells were then shown to be effectively seeded onto prosthetic graft surfaces and exhibit long term and high efficiency viability through the seeding process (p. 15).

40. Prosthetic vascular implants prepared according to the methods of the invention are further shown in the specification to be useful, *inter alia*, for implantation into mammalian (baboon) subjects (pp. 15-19). After implantation, the grafts were demonstrated to remain patent (e.g., p. 16, lines 27-32) and to express the exemplary transgene on a long-term basis (e.g., 3-5 wks; p. 17-19, p. 18, lines 14-20). These results were the subject of a peer-reviewed publication entitled "Gene Transfer in Baboons Using Prosthetic Vascular Grafts Seeded with Retrovirally Transduced Smooth Muscle Cells: A Model for Local and Systemic Gene Therapy" (Geary et al., Hum. Gene Ther. 5:1211-1216, 1994 (of record).

41. The unique mode and route of delivery of the invention, using *in vitro* transduction and local engrafting of smooth muscle cell-seeded grafts provides unexpected advantages over systemic gene/vector delivery, the latter of which has now been determined to expose vectors to inactivation by serum factors prior to the infection of target cells. For example, vector inactivation in skin fibroblasts has been documented in both rats (Palmer et al., 1991) and dogs (Ramesh et al., 1993). In contrast, further evaluation of the claimed devices and methods of the invention has demonstrated that retroviral vector sequences are not inactivated by SMCs seeded onto vascular grafts.

42. The presently claimed invention overcomes yet additional weakness of direct, *in situ* transduction methods, including poor safety and definition relating to *in vivo* targeted cell populations (e.g., based on uncertain vector delivery, infection success, targets and extent of tissue transduction, level of gene expression—all of which are less manageable and predictable in direct, *in situ* methods compared to the present methods). One particularly important safety feature of the invention relates to the localization and control of transduced cell SMC populations in implanted

grafts. As described in the specification, seeded, transduced SMCs are efficiently retained in the implanted grafts (see, e.g., p. 19, lines 1-7). Subsequent experiments validate this disclosure by demonstrating that there is no detectable presence or persistence of dislodged, transduced SMCs persisting in long-term graft recipients (rats) that could impair the safety of the grafting procedures.

43. While it is understood that therapeutic efficacy is not a requirement to establish patentability of the presently claimed subject matter, the results demonstrated for the invention in this regard nonetheless emphasize the nature "unexpected results" provided by the invention. As expressly forecast in the specification, vascular grafts according to the present invention have been shown to be capable of delivering therapeutic levels of multiple, highly relevant transgenes in accepted model hosts. Thus, the record provides evidence establishing *in vivo* expression of therapeutic levels of granulocyte-colony stimulating factor (G-CSF) manifesting sustained, therapeutic increases in neutrophil levels in canine subjects, as well as rats, using the claimed SMC-engrafting devices and methods (see also, Applicants' April 2, 1996 amendment and supporting exhibits).

44. Referring to the data developed in a canine model system, efficient transduction and seeding of G-CSF-expressing SMCs on prosthetic grafts implanted in canine subjects has been demonstrated and published (see, e.g. Osborne et al., Clin. Res. 41: 194A, 1993, of record). In this study which follows the general teachings of the specification, the seeded grafts were removed after three months of *in vivo* activity and yielded healthy transduced cells that secreted G-CSF in culture (id.). During the course of graft implantation, the test subjects exhibited significant, therapeutically relevant increases in neutrophil production. These clinical data correlated with G-CSF expression from the implanted, seeded PTFE grafts. In particular, neutrophil levels increased from control levels of 5,000-6,000 PMN/microliter to post-treatment levels of 8,000-9,000 PMN/microliter, after three months of treatment.

45. As further demonstrated in another peer-reviewed publication that followed the teachings of the specification (Osborne et al., Proc. Natl. Acad. Sci. USA 92: 8055-8058, 1995; Exhibit 2 to Applicants April 2, 1996 Amendment), long-term expression of EPO in rats has been achieved using transduced vascular smooth muscle cells seeded on vascular grafts for greater than

seven weeks, and subsequent data have been obtained for expression out to greater than nine months. As noted in this publication:

> These data indicate a relatively efficient seeding procedure that results in a cell mass capable of providing sustained gene delivery at therapeutically significant levels. [p. 8057; col. 1, first para; emphasis added.]
>
> ***
>
> The constitutive level of Epo we achieved in this study would provide useful therapy for patients with renal failure. Although arterial seeding is not feasible in human subjects, we have recently shown in baboons that prosthetic vascular grafts can be used as a device to implant transduced cells. From the data produced in this rat model and our studies in dogs and baboons, we estimate that $10^8$ transduced vascular smooth muscle cells can provide a therapeutic dose of Epo to an 80-kg patient, and this cell number could be transplanted in a 10 cm x 4 mm prosthetic graft.... The ability to treat these patients, and others with Epo-responsive anemias, by gene therapy would provide major clinical and economic benefits. [p. 8057, col. 2, lines 13-30; citations omitted; emphasis added.]

46. It should be further noted in this context that the invention allows modulation of delivery of EPO and other therapeutic products to achieve therapeutic levels of delivery, unrealized by other modes and routes of gene therapy. In fact, the amount of erythropoietin that is secreted per cm. of graft containing transduced cells can be pre-determined to achieve a desired hematocrit, e.g., by altering the length of grafts or density of seeded cells.

47. Thus, the record provides evidence of long-term, adjustable, therapeutic levels of erythropoietin (EPO) expression manifested by therapeutically relevant increases in hematocrit levels in rats and dogs using the engrafting devices and methods of the invention. Additional data has been developed in baboons which support adjustable delivery of EPO in these subjects, based on seeded cell number and graft size. Presently, the inventors' have received approval from the University of Washington Human Subjects Committee (IRB) to implant PTFE dialysis access grafts seeded with transduced SMCs expressing human EPO into patients with end-stage renal disease(ESDR).

47. Thus, the record provides evidence of long-term, adjustable, therapeutic levels of erythropoietin (EPO) expression manifested by therapeutically relevant increases in hematocrit levels in rats and dogs using the engrafting devices and methods of the invention. Additional data has been developed in baboons which support adjustable delivery of EPO in these subjects, based on seeded cell number and graft size. Presently, the inventors' have received approval from the University of Washington Human Subjects Committee (IRB) to implant PTFE dialysis access grafts seeded with transduced SMCs expressing human EPO into patients with end-stage renal disease(ESDR).

48. The foregoing evidence clearly demonstrates that the devices and methods of the invention, as described in the specification and as set forth in the pending claims, provide "unexpected results" over the prior art of record, viewed for what it teaches as a whole (as discussed above). In particular, even if it is considered that the art "suggests" to make the devices of the invention as claimed, there is no reasonable expectation of success that can be gleaned from these references to obtain the disclosed results, on an individual or collective basis.

49. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize validity of the application or any patent issuing thereon.

Date: 3/7/01 By: WRA Osborne
William R. A. Osborne

APPENDIX

CURRICULUM VITAE
OF
William R. A. Osborne, Ph.D.

Personal Data

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Education

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| Undergraduate: | 1957-1965 | Plymouth University, Plymouth, England<br>Graduate, R.I.C., Chemistry |
| Graduate: | 1968-1969 | University of Bristol, Bristol, England<br>M.Sc., Advanced Analytical Chemistry |
| | 1969-1972 | King's College, University of London, England<br>Ph.D., Biochemistry |

Positions Held

| | |
|---|---|
| 1957-1968 | Analytical Chemist, Central Electricity Generating Board, England |
| 1969-1972 | Part-time teaching, North East London Polytechnic, Barking, England |
| 1972-1975 | Postdoctoral Fellow, Department of Human Genetics, University of Michigan School of Medicine, Ann Arbor, Michigan |
| 1975-1976 | Research Associate, Department of Pediatrics, University of Washington School of Medicine, Seattle, Washington |
| 1976-1981 | Research Assistant Professor, Department of Pediatrics, University of Washington School of Medicine, Seattle, Washington |

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|---|---|
| 1981-1990 | Research Associate Professor, Department of Pediatrics, University of Washington School of Medicine, Seattle, Washington |
| 1990-Present | Research Professor, Department of Pediatrics, University of Washington School of Medicine, Seattle, Washington |
| 1995-Present | Director of Gene Therapy Research, Center on Human Health and Disability, University of Washington. |

Memberships

Biochemical Society, London, England
Royal Institute of Chemistry, London, England
American Association for the Advancement of Science
American Society of Gene Therapy

Honors and Awards

Scientific Editorial Board Human Gene Therapy 1992-to date

Henry Christian Memorial Award for Excellence in Clinical Research, American

Federation of Clinical Research 1992
Member Medical Biochemistry Study Section, DRG, NIH, 1993-1997
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